# CHROMOSOME NUMBERS AND KARYOTYPES OF SOME AUSTRALIAN STIGMODERINI (COLEOPTERA: BUPRESTIDAE)

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#### Summary

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Karyotypes of eight species of Australian Stigmoderini are iliustrated and compared Stigmodera (S.) goryi Gory & Laporte, S. (S.) porosa Carter, S. (Themographia) donovani Gory & Laporte, S. (T.) heros Gehin, S. (T.) tricoloraça Waterhouse and S. (T.) viridicineta Waterhouse have a diploid complement of 22; S. (T.) alternata Lumholtz and S. (T.) mekerli Obenberget have 2n = 20. A chromosome number of 2n = 20 is reported for 26 additional species. All Stigmoderini studied have an  $Xy_p$  sex determining mechanism.

KEY WORDS: Coleoptera, Buprestidae, Stigmoderini, Chromosomes.

#### Introduction

Coleopteran cytogenetics was pioneered by Stevens in the first decade of the twentieth century, and two species of buprestids, un-named spruce borers, were among the earliest examined (Stevens 1906). Smith & Virkki (1978) listed 22 species of buprestid, whose diploid chromosome number ranged from 12-26.

The Xy<sub>p</sub> association is one in which the y is very small, approaching the lower limit of visibility. The X and y form a characteristic ring bivalent which Stevens (1906) described as a "parachute" in which the X chromosome represents the parachute proper, and the y represents the load. It is the commonest system in Polyphaga, occurring in practically all families, and Smith (1950) adopted the symbol Xy<sub>D</sub>.

This study was undertaken as part of a revision of the tribe Stigmoderini. The aim was to determine it karyological characters could contribute to an assessment of the higher categories as delineated on the basis of morphological characters.

#### Materials and Methods

Adult male specimens were dissected as soon as possible after capture. Based on the technique of Imai et al. (1977) the testes were treated with a cold hypotonic (1% sodium citrate solution) for 1 h, then 0.005% w/v colchicine in hypotonic for 15 min before fixation in 3:1 ethanol: acetic acid for 30 min. The cells were spread and air-dried before staining with 10% Giemsa in Sörensen buffer at pH 6.8 for 15 mins. Photomicrographs were taken on a Zelss Photomicroscope Model III at magnification 400x, using a green filter and Agfa-Gevaert Copex Pan Rapid Tri 13 film. Chromosome preparations and

corresponding specimens are lodged in the South Australian Museum, Adelaide

Chromosome counts were obtained for the following species: Stigmodera (Stigmodera) cancellata (Donovan), S. (S.) goryi Gory & Lapurte, S. (S.) gratiosa Chevrolat, S. (S.) macularia (Donovan), S. (S.) porosa Carter, S. (S.) roei Saunders, S. (Themognatha) alternaty Lumboltz. S. (T.) barbiyentris Carter, S. (T.) bonyouloiri Saunders, S. (T.) chalcodera Thomson, S. (T.) chevrolati Gehin, S. (T.) donovani Gory & Lapone, S. (T.) heros Gehin, S. (T.) mitchelli Hope, S. (T.) mniszechi Saunders, S. (T.) nickerli Obenberger, S. (T.) parvicollis Saunders, S. (T.) pubicollis Waterhouse, S. (T.) regia Blackburn, S. (T.) tricolorata Waterhouse, S. (T.) variabilis (Donovan), S. (T.) viridicineta Waterhouse, S. (Castiarina) adelaidae Hope, S. (C.) argillacea Carter, S. (C.) cupreoflava Saunders, S. (C.) decemmuculata (Kitby), S. (C.) flavopicta (Boisduval), S. (C.) gratu Saunders, S. (C.) rufipennis (Kirby), S. (C.) sexplagiata Gory, S. (C.) simulata Gory & Laporte, S. (C.) subnotata Carter, S. (C.) subtincta Carter, and S. (C.) triramosa Thomson,

Between one and three karyotypes were made from mitotic metaphase spreads of 17 species, and where more than one was made, there was good agreement, to the nearest percentage, between the relative total chromosome lengths (TCL) and arms, as measured from the enlarged photographic prints. Karyotypes are assigned formulae following Smith (1965).

#### Results

Of the 34 species examined, S. nickerli and S. alternata have a diploid complement of 20, the rest have 2n = 22. All have an  $Xy_p$  sex-determining mechanism (Figs 1, 2). Karyotypes of 17 species are summarized in Table 1, and eight of these are illustrated in Fig. 3

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Autosomes are predominantly metacentric, but some of the karyotypes analysed have acrocentrics e.g. S. nickerli (autosome 9), S. viridicincta (autosomes 7 and 10), or submetacentrics e.g. S. tricolorata (autosome 10). Changes in arm ratio may be due to pericentric inversion, changes in heterochromatin, or reciprocal translocation (Imai et al. 1977). Karyotype variations due to pericentric inversions in congeners are known in many genera of beetles (Yadav & Pillai 1979).

Of the eight species whose karyotypes are illustrated, six (S. porosa, S. nickerli, S. tricolorata, S. goryi, S. alternata, and S. donovani) have an autosomal pair with a nucleolar organizer region (NOR) (Figs 3a, b, d, e, g, h). When present, it is usually on autosome 8, or one of the adjacent chromosomes which are often so close in length that it is difficult to order them exactly. The position of the NOR is either pro-centric on the long arm as in S. porosa, or subterminal as in S. nickerli and S. alternata. In the latter cases, the distal ends of the arms form satellites.



Fig. 1. Late male meiotic metaphase I of Stigmodera (S.) gratiosa, arrow indicates Xy<sub>p</sub> bivalent.

In S. nickerli and S. alternata, autosome 1 is approximately twice the length of autosome 2, and represents about 22-23% TCL (Table 1). In the other Stigmoderini, autosome 1 varies from 1-1.5 x length of autosome 2 and represents only 12-15%

TABLE 1. Karyology of 17 species of Stigmoderini. A = autosomes; superscripts m, sm, sa, a, represent metacentric, submetacentric and acrocentric respectively; S, M, L = small, medium and large size of X chromosome relative to autosomes; A1% = length of autosome 1 expressed as a % TCL; NOR = autosome number on which NOR occurs, where several autosomes are the same length so that the exact order cannot be determined, the number is given as a group; — indicates that no NOR was discerned.

Taxon	Formula	X	A1%	NOR
Stigmodera (Stigmodera)				
cancellatu	$10 A^m + X^m y_p$	S	17	_
goryi	$10 \text{ A}^{\text{in}} + \text{ X}^{\text{sm}}\text{y}_{\text{p}}$	S	13	7-8
grutiosa	10 A + Xy <sub>p</sub>	S	13	_
macularia	$10 \text{ A} + \text{X}^{\text{sm}}\text{y}_{\text{p}}$	L	18	- manual-
porosa	$10 \text{ A}^m + X^{\text{say}}$	L	18	7-8
roei	10 A <sup>m</sup> → X <sup>sm</sup> y <sub>p</sub> .	L	16	_
S. (Themognatha)	- p			
alternata	9			
	$A^m + X^m y_n$	S	22	8
barbiventris	$10 \text{ A}^m + \text{ X}^d \text{y}_n$	S	15	_
chevrolati	7			
	$A^{m}$ + 3 $A^{5m}$ + $X^{5m}y_{n}$	S	13	
donovani	10 A + X <sup>5m</sup> y <sub>p</sub>	S	14	6-8
heros	10 Am - Xsay <sub>n</sub>	L	15	_
mitchelli	9			
	$A^m \cdot I A^{sa} + X^a y_p$	S	12	_
nickerli	8			
	$A^m - 1 A^a + X^{5m} y_D$	S	23	8
reglu	9			
	$A^m + 1 A^a + Xy_p$	M	12	_
tricolorata	9			
	$A^{m} \rightarrow 1 A^{sm} + X^{sa}y_{p}$	-5	13	7-8
cariabilis	8			
	$A^{m} \rightarrow 2 A^{sm} + X^{a}y_{p}$	S	13	7-9
VIridicineta	8			
	$A^{m} + 2 A^{a} + X^{a}y_{n}$	S	15	

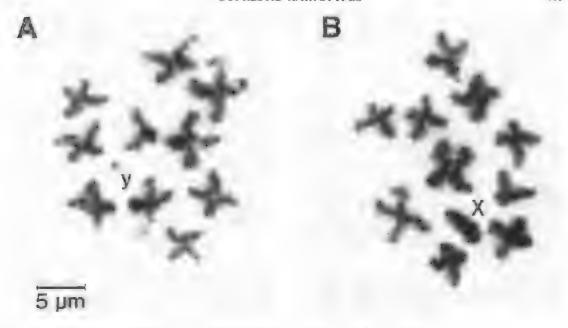


Fig. 2. Male meiotic metaphase II of Stigmodera regla (A) 10 A + y; (B) 10 + X.

TCL S. (Themognatha), or 13-18% TCL Stigmodera (sensu stricto).

The most distinctive difference between species is the relative size of the X chromosome (see Table 1). In some species e.g. S. porosa and S. heros, the X approaches the largest autosome in length, but in most it is one of the smallest. In the majority of species the arms of X are unequal, often markedly so, and the X is heterochromatic as indicated by both the differential staining and its diphasic form. A chromosome appears diphasic when the euchromatic arms condense earlier and the split between their chromatids becomes clearly visible, whereas the condensation of the heterochromatic arms proceeds more slowly and the chromatids remain jointly coiled for a longer time (Smith & Virrki 1978).

#### Discussion

The study of 34 species of Stigmoderini indicates generic stability of chromosome number. The limited data on other buprestids support this e.g. the four species of Agrilus studied by Smith (1949, 1953) have 20 or 22; the three species of Dicerca reported have 20 (Smith 1953), and two species of Sternoceru have 26 (Asana et al. 1942).

The differences in chromosome number of S. (T.) nickerli and S. (T.) alternata, with  $2\pi = 20$  may be due to Robertsonian rearrangements. Centric fusion or fission are suggested when there is a change in

the number of chromosomes, but not in the total number of major chromosome arms (the fundamental number). The centric fusion of two acrocentric autosomes such as 7 and 10 of S viridcincta could have given rise to a karyotype such as S. alternata with nine metacentric autosomal pairs. The fused chromosomes would approximate in size the large relative length of chromosome 1.

On the other hand, translocations have played an important role in the karyotype evolution of beetles (Virkki 1984). The large size of autosome 1 in S. nickerli and S. alternata (22% TCL) may have evolved from a karyotype similar to the other Stigmodera by translocation of one of the smaller autosomes (6-9% TCL) on to autosome 1 (12-18%), followed by a pericentric inversion which resulted in the new autosome 1 reverting to a metacentric.

The preponderance of species with  $2n-20+Xy_p$  in Stigmoderini suggests that 22 chromosomes may be the ancestral condition of the tribe, with the complements of S. nickerli and S. alternata being apomorphic.

The Xy<sub>p</sub> sex-determining mechanism has been recorded in ten of the 21 species of Buprestidae reported by Smith & Virkki (1978), the others had XY, Xy, neo-XY or XO. Crowson (1981) maintained that it is the most primitive condition, and is suspected to have been a feature of the ancestors of the Endopterygota at the beginning of the Permian period, although this question is still under

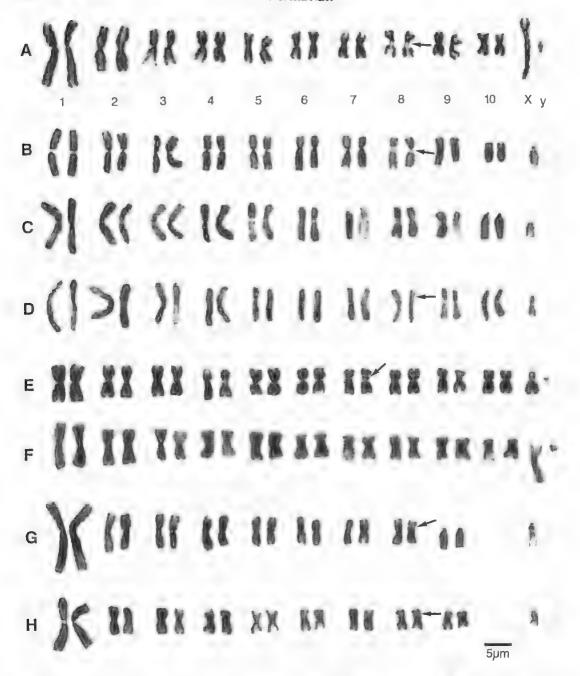


Fig. 3. Male karyotypes derived from mitotic metaphase plates. (A) Stigmodera porosa; (B) S. tricolorata; (C) S. viridicineta; (D) S. donovani; (E) S. goryi; (F) S. heros; (G) S. nickerli; (H) S. alternata. Arrows indicate NORs, all karyotypes are at the same scale.

debate. Opinions about the mechanism of pairing  $X_p$  and  $y_p$  have oscillated between nucleolar and chiasmate modes and there is now evidence for both associations (Virkki 1984).

It is probable that the variation in relative size of the X chromosome is due to the duplication or deletion of constitutive heterochromatin which could occur without deleterious effects. Structural

alterations such as translocations also may have been responsible for some of the variation observed. Variations in the X chromosome do not correspond to species groups formed on the basis of other characters (Gardner 1986).

Crowson (1981) asserted that chromosomal features of Coleoptera do not provide very reliable characteristics of taxa at higher levels, though he noted two exceptions: superfamily Cantharoidea in which all 25 species studied have an XO sexdetermining mechanism; and superfamily Curculionoidea in which the karyotypes of all species studied are derived from a basic ten autosomal pairs, not nine. Blackman (1980) in his study of 180 species of Aphidae expressed a similar opinion, although he found that in general, chromosome data corroborate generic concepts since there is a clear tendency for the chromosome number to be stable at this level, and that differences in chromosome numbers sometimes agree with recognized subgeneric groupings.

Yadav & Pillai (1979) in their study of phylogenetic relationships of genera and subfamilies of Scarabaeidae, placed considerable importance on chromosome number which they found varies from n=6-11, with 150 of the 194 species having the modal number of n=10. They considered the two tribes Adorrhinyptiini and Adoretini to be closely related because they both have 2n=22. However, the occurrence of 2n=22 in two genera from other subfamilies, Geotrupes and Dynamopus, not considered taxonomically close on other grounds was considered to be parallelism,

My exploratory chromosome studies of Stigmoderini support the general observation of generic stability in chromosome number and suggest that karyology may he useful in delimiting species groups, S. nickerli and S. alternata which both have 20 chromosomes are morphologically very similar. The use of C-banding techniques when more material becomes available could provide insights into the relationships between the complements of 20 and 22, the evolution of the acrocentrics, and the evolution of the X chromosome.

Coleopteran cytogenetics is still in its infancy because of the small size of the chromosomes and associated problems of obtaining high quality karyotypes, so perhaps it is too carly to assess its use in systematics. Uniformity of many of the gross chromosomal features supports the naturalness of Stigmoderini as a group, but gives less scope for elucidating relationships within the tribe. More detailed karyological analyses in the future may provide valuable phylogenetic information, and be useful at the level of species group.

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# A NEW SPECIES OF *NOTOPLAX* (MOLLUSCA: POLYPLACOPHORA: ACANTHOCHITONIDAE), FROM NEW SOUTH WALES, AUSTRALIA

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# **Summary**

A new species, *Notoplax lancemilnei* sp. nov., is described from deep water off central New South Wales, Australia. It most closely resembles *N. speciosa* Adams but is distinguished from it by the larger, more irregular tegmentum pustules, ridges filling in part of the insertion plate grooves, and by its colour. The new species was trawled by the F.R.V. "Kapala" in 400-500 m of water. KEY WORDS: Chiton, Polyplacophora, Acanthochitonidae, New South Wales, Australia, *Notoptax*, new species